



# Ready To Use PCR Reagents

## Feline *Bartonella henselae*

Cat. No. 60FBH100

### INSTRUCTION MANUAL

#### I. Intended Use

**FBH Ready to Use PCR Reagents** are intended for Feline *Bartonella henselae* amplifications. All reagents are ready to use for a successful amplification, from DNA extraction to obtaining PCR products suitable for loading onto the Agarose gel.

#### II. General Information

Each package contains **Rapid One Step Extraction Buffer** (Tube A), which is intended for use with fresh or dry blood samples. The extraction step yields appropriate amount of crude DNA needed for a successful amplification of FBH via PCR. No purification is needed! Tubes B, C, and D are the components for subsequent use in PCR amplification. Tube B contains **FBH-PCR mix**, Tube C contains **FBH Activation Buffer** and Tube D contains the **Positive Control**. The Extraction Buffer (Tube A) also serves as **Negative Control**. Also included are **Tissue/Swab Extraction Buffer** (Tube E) and **Tissue/Swab Neutralization Buffer** (Tube F). Each PCR set up should include 3 reaction vials; each vial should be added with: **5µl FBH-PCR mix**, **10µl FBH Activation Buffer** and **5µl DNA product of the Extraction step / Positive Control/ Negative Control**. Following the addition and mixing of all the above ingredients, the reaction vials are placed in thermal cycler for amplification according to the program detailed in the Step by Step chapter (see section VIII). At the end of the program the product should be visualized on 1.5% Agarose gel, yielding a **220bp** band.

#### III. Description Of The Disease

The genus *Bartonella* is currently comprised of at least 20 species and subspecies of vector-transmitted, fastidious, gram-negative bacteria that are highly adapted to one or more mammalian reservoir hosts. *Bartonella henselae* (FBH) cause persistent intravascular infection in domestic cats and wild felid species. Self-limiting fever can also occur in FBH bacteremic cats following minor surgical procedures. Cats experimentally infected with FBH show self-limiting febrile illness of 48–72 hours duration, mild to moderate transient anemia, and transient neurologic dysfunction. Bacteremia caused by FBH had been documented in 4–41% of healthy cats in different regions throughout the world. The main concern regarding FBH is its association with human fever and lymphadenopathy termed Cat Scratch Disease, which may be fatal in immunocompetent population worldwide distribution.

#### IV. Diagnosis Of The Disease

Serum antibody tests alone cannot be used to document clinical uveitis associated with FBH infection; cats may have FBH antibodies regardless of recent infection, while 2% of FBH infected cats lack antibodies. Diagnosis of FBH infection should be confirmed by culturing the organism from blood or tissues such as lymph node or heart valve, or by amplifying *Bartonella*-specific DNA sequences using PCR. PCR from blood and tissue samples is far more rapid than culturing and has been shown to be highly sensitive, making it the preferable diagnostic method.

#### V. Contents (Sufficient for 48 tests)

Tube A	Rapid One Step Blood Extraction Buffer
Tube B	FBH-PCR mix (Green cap)
Tube C	Specific FBH Activation Buffer (Blue cap)
Tube D	Specific FBH Positive Control (Red cap)
Tube E	Tissue/Swab Extraction Buffer
Tube F	Tissue/Swab Neutralization Buffer
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#### VI. Essentials Not Included

RNAase free PCR reaction vials.  
PCR Thermo-Cycler.  
5-10µ, 100µl Pipettes and filter tips.  
Micro-centrifuge.  
Heating bath or heating block.  
Agarose, DNA size marker.  
Microwave for Agarose casting.  
Horizontal Mini-Electrophoresis chamber, Comb and power pack.  
TBE /TAE Buffer and Ethidium Bromide (EB).  
UV Transilluminator (254nm for EB).  
A pair of sterile scissors.  
A cutter (for swab application).

#### VII. Storage And Handling

- Store at 4°C for 6 months or at -20°C for two years.
- Use gloves and maintain clean working conditions.
- Avoid spillage and cross contamination of solutions.
- Change tips between reagents and between reaction vials.
- Disinfect scissors before and after each cutting blood filters.
- Do not mix reagents from different batches.
- Always treat samples with precaution, and dispose as biological material.
- Remember that Ethidium Bromide is hazardous, and use the UV transilluminator carefully.
- It is recommended to incinerate the contents after use.

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## VIII. Step By Step Protocol

### Blood Extraction:

- (1) Into an empty clean vial, add **100µl of Rapid OneStep Blood Extraction Buffer (Tube A)** for **every 5µl** of fresh blood sample or approximately 3/5 mm<sup>2</sup> piece of Whatman/tissue paper soaked with blood. Make sure the piece of paper is submerged underneath the extraction buffer.
- (2) Incubate samples at **50°C** for **10 minutes** followed by a subsequent **95°C** for additional **10 minutes**.
- (3) Centrifuge sample at **>10,000 rpm** for **1 minute** to allow the paper and cell debris to pellet. The extracted DNA product is in the liquid phase, ready to be used for PCR.

### Tissue/Swab Extraction:

- (1) Into a clean 1.5 ml vial add **300µl of Tissue/Swab Extraction Buffer (Tube E)**.
- (2) When using tissue sample, cut a 3 mm<sup>2</sup> from the fresh or frozen tissue and add it to the 1.5 ml vial containing **300µl of Tissue/Swab Extraction Buffer**.
- (3) Incubate the tissue within buffer **E** for **10 minutes at 95°C**.
- (4) Add **300µl of Tissue/Swab Neutralization Buffer (Tube F)** and the product will be ready for PCR use.

Extracted DNA product (of any source)\* may be applied immediately for PCR or stored for a few days at 4°C / several months at -20°C. Please mark the vial properly for future identification.

\* Note: **The reagents have been adjusted for use with crude DNA extraction to enable better sensitivity (with less handling).**

### PCR Procedure:

- (1) Into a clean reaction vial add: **5µl FBH-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific FBH-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.
- (2) Into a second clean reaction vial add **5µl FBH-PCR mix (Tube B)**, **5µl of the Positive Control (Tube D)** and **10µl of the specific FBH Activation Buffer (Tube C)**. Mark this vial as Positive Control reaction.
- (3) Into a third clean reaction vial add **5µl FBH-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific FBH Activation Buffer (Tube C)**. Mark this vial as **Negative Control** reaction.
- (4) Gently mix each reaction vial (do not vortex!) and place in the thermal cycler for amplification.

### PCR Program:

**A. 95°C for 2 minutes**

**38 cycles of:**

**B. 94°C for 30 seconds**

**C. 53°C for 30 seconds**

**D. 72°C for 30 seconds**

**End cycles**

**E. 72°C for 2 minutes**

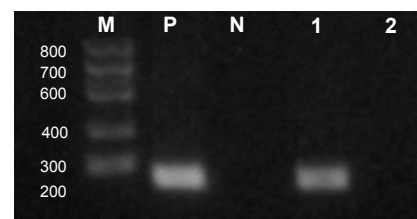
**F. Stop at 8°C**

- (5) If not used immediately, store PCR products at 4°C until application on Agarose.

## IX. Reading And Interpreting The Results

- Visualize PCR products on 1.5% Agarose gel, along with a size marker (see Fig. 1).
- Mark each well and load the whole content of each reaction vial into the relevant wells.
- The Positive Control should yield a single band at **220bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **220bp**.

**Fig. 1 - Visualization of the PCR product.**



Lanes: M Size Marker, P Positive Control, N Negative Control  
Lanes 1 - 2 are test samples of which 1 is positive for FBH.

## X. Limitations of the kit and troubleshooting

- For *in vitro* use only. Do not use internally or externally in humans or animals.
- A false positive result may occur, even if precaution has been taken. To eliminate inconclusive results, always use the Negative Control in each PCR set.

## XI. References

- JBoulouis HJ et al. (2005) Factors associated with the rapid emergence of zoonotic Bartonella infections. Vet. Res. 36: 383–410.
- Kordick et al. (1999) Clinical and pathologic evaluation of chronic Bartonella henselae or Bartonella clarridgeiae infection in cats. J. Clin. Microbiol. 37:1536–1547.
- Lappin MR et al. (2000) Bartonella spp antibodies and DNA in aqueous humour of cats. J Feline Med Surg. 2(1):61-8.
- Maruyama S et al. (2001) Prevalence of Bartonella species and 16s rRNA gene types of Bartonella henselae from domestic cats in Thailand. Am. J. Trop. Med. Hyg. 65: 783–787.

For further information and assistance please contact your local distributor or Biogal Galed Labs. Directly by e-mail: info@biogal.co.il or by tel: 972-4-9898605 / fax: 972-4-9898690.